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Determination of Hydantoins in Condensate Water from Lignite Gasification

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A method for the characterization of the nonsolvent extractable hydantoins in condensate water from the slagging fixed bed gasification of lignite was developed. The water was treated with activated carbon and the hydantoins were extracted from the carbon with boiling alcohol. A number of 5,5-dialkyl- and 5-alkylhydantoins were separated by gas chromatography on a polar fused silica capillary column and characterized by comparison of their mass spectra with known standards and by matching retention indexes. For compounds with low intensity molecular ions, chemical ionization with methane was used for identification. The 1,3-dimethyl derivatives of the hydantoins were prepared and chromatographed on a nonpolar capillary column to provide further confirmation of the presence of the hydantoins.

The existence of polar nonsolvent extractable compounds in the condensate water from the slagging fixed bed gasification of lignite has been known for some time. Only recently have the major constituents of the condensate water been identified (*1*, *2*). This paper describes the characterization of the various alkylhydantoins found in water from the GFETC gasifier.

Condensate water which had been extracted three times with methylene chloride was treated with activated charcoal. The filtered charcoal was then extracted with boiling ethanol. Evaporation of the ethanol gave a white solid in a yield of 1.5 g/L of condensate water. The white solid was analyzed by gas chromagraphy, using a polar wall-coated phase (Superox-FA, AT-1000) fused silica capillary column. GC/MS data were obtained in both EI and CI modes for the components of this mixture from the ethanol extraction of the charcoal. Retention index matching with reference hydantoin standards was carried out with a flame ionization detector. Dimethyl derivatives which exhibit more intense molecular ions than the underivatized hydantoins were prepared and analyzed by GC/MS on a nonpolar capillary column.

EXPERIMENTAL SECTION

The condensate water was produced from gasification of Indian Head lignite in the GFETC slagging fixed bed gasifier at 300 psig with oxygen feed rates of 6000 scfh (standard cubic feet per hour) and an oxygen to steam molar ratio of 0.9 to 1.0 (*3*). The product gas was cooled from about 160 to 50 °C in a spray washer, which circulates the condensed water into contact with the hot gas. After a residence time of about 2 h, the liquor was fed to a tar water separator (50 °C) where the water had a residence time of 6-7 h.

Condensate water (500 mL) was stirred for 2 h with 10 g of charcoal (Pittsburgh Activated Carbon, Calgon 12 × 40) which had been ground in a mortar and pestle. The charcoal was filtered by gravity and the pH of the filtrate was adjusted to 2.0 by adding HCl. The acidified filtrate was stirred with a second 10 g of charcoal. Filtration was again performed and the charcoal was combined with that obtained earlier and extracted with boiling ethanol (300 mL) for 30 min. Evaporation of the ethanol on a rotary evaporator gave a residue. If the condensate water had been previously extracted with methylene chloride, diisopropyl ether, or methyl isobutyl ketone, the residue contained only a very small amount of phenols and weighed about 0.75 g. If unextracted condensate water was treated with charcoal, then 2.5-3 g of phenolics was also present in the residue.

A Hewlett-Packard 5880A gas chromatograph with FID detection and on-column injection (0.2 μL) was used for the retention index studies. The column was a 25-m Superox-FA (AT-1000) fused silica capillary with 0.32 mm i.d. and 0.3-μm film thickness. The carrier was ultrahigh-purity hydrogen passed through an oxygen trap and a molecular sieve–Drierite tube and set at a flow rate of 2 mL/min (41 cm/s) at 260 °C. Nitrogen was used as detector make-up gas. The residue or hydantoin standards were dissolved in methanol.

Multilevel oven temperature heating rate program was as follows: initial temperature, 60 °C; program heating rates, 30 °C/min to 85 °C, 0.5 °C/min to 100 °C, 1 °C/min to 200 °C, and 1.5 °C/min to 260 °C.

Although a multilevel heating rate program was used, the retention indexes remained constant as long as the head pressure on the column was not changed.

The GC/MS data were obtained with a Hewlett-Packard 5985B and a 25-m Superox-FA (0.25 mm i.d.) fused silica capillary column (on-column injection) for the underivatized hydantoins. Hydrogen carrier at 1 mL/min was used. Electron energies of 70 eV for EI and 200 eV for CI modes were used. Source temperature was 200 °C, scan rate was 267 amu/s, and mass range was 40-400 amu. Methane was the CI reagent gas.

5,5-Dimethylhydantoin was purchased from Aldrich Chemical Co. Other hydantoins were synthesized from the cyanohydrin by the method in Vogel (*4*).

Methylation of the hydantoins was carried out by use of methyl iodide in a pH 13 buffer (*5*). A 60-m DB5 fused silica capillary

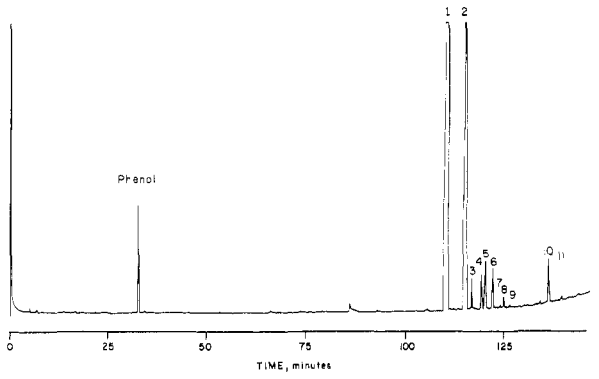


Figure 1. Gas chromatogram of solids obtained by charcoal treatment of solvent-extracted condensate water. Conditions are specified in the Experimental Section, detector FID. Numbered chromatographic peaks are identified in Table I.

column was used for chromatography of the methyl derivatives.

RESULTS AND DISCUSSION

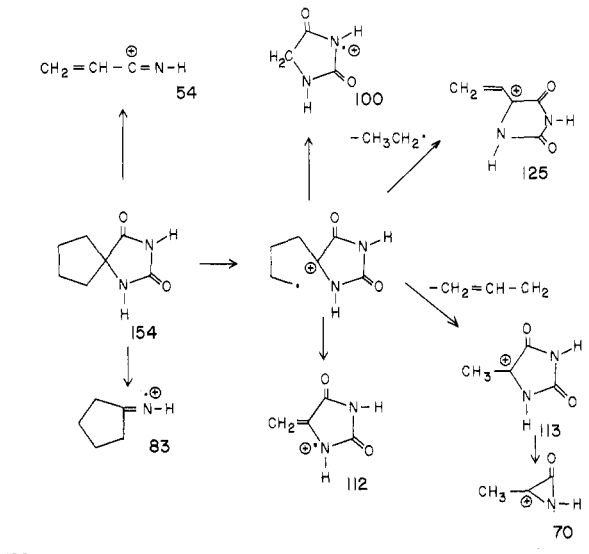
Gas chromatography with the poly(ethylene glycol) phthalate phase (Superox-FA) in a fused silica capillary column gave good peak shapes (very little tailing) and excellent resolution of the components. A multilevel oven heating rate program was used which flash vaporizes the methanol solvent and then increases slowly enough to effect adequate resolution of phenolics as well as the hydantoins which elute much later. Two major peaks and several smaller peaks were observed at retention times (see Figure 1) considerably longer than that of the small quantity of phenol which was also present in the mixture obtained from the charcoal.

GC/MS studies with the same GC column were performed on the mixture of nonsolvent extractables isolated from the charcoal treatment. The largest component (peak 1, Figure 1) exhibited a molecular ion peak at m/e 128, with a base peak at 113 (M - 15). The mass spectrum (EI mode) closely matched that of 5,5-dimethylhydantoin. Masses below 50 were omitted because of the high background from column bleed associated with this phase. Cleavage of the hydantoin ring to give ions at m/e 100 (loss of CO), 70 (loss of HNCONH or CH_3 and NHCO), and 57 (loss of CO plus NHCO) are consistent with fragmentation reported previously for 5,5-dimethylhydantoin (*6*).

The molecular ion for the second largest peak (peak 2, Figure 1) was observed at m/e 142, with a base peak at 113. This spectrum matched that of 5-ethyl-5-methylhydantoin. Loss of the methyl group from the molecular ion gave a low intensity ion (relative abundance 1.7%) at 127. In this spectrum, the m/e 114 ion was observed for the fragment resulting from either loss of CO or the McLafferty cleavage of the ethyl group with tranfer of the γ-hydrogen (*6*, *7*). Fragments were observed with m/e 71 from the loss of CO and NHCO from the molecular ion, with m/e 70 from the loss of NHCO from the 113 ion, and with m/e 56 from loss of CO and NHCO from the 127 ion (*6*).

Some of the hydantoins present in smaller concentrations could not be identified on the basis of EI GC/MS data obtained from underivatized hydantoins separated on the Superox-FA column because of very low molecular ion intensities. The components corresponding to peaks 3, 4, and 5 on the chromatogram (Figure 1) fragmented to give ions at m/e 113 and 114 in high intensity and at 127, 128, and 141 in lower abundance. These patterns would be expected for the diethyl- and methylpropylhydantoins; however, further work using CI mode, derivatization, and retention index matching was necessary to characterize these components.

5-Methylhydantoin exhibited a molecular ion at m/e 114 and fragment ions at 99, 86, and 71 (*6*, *7*). These ions were



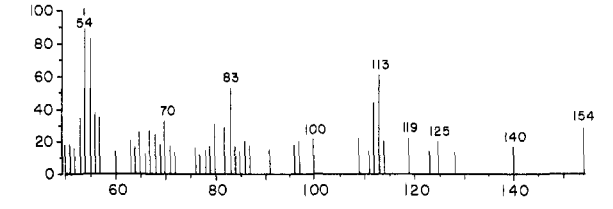


Figure 2. Mass spectrum (EI) and fragmentation scheme for spiropentylhydantoin (peak 10, Figure 1).

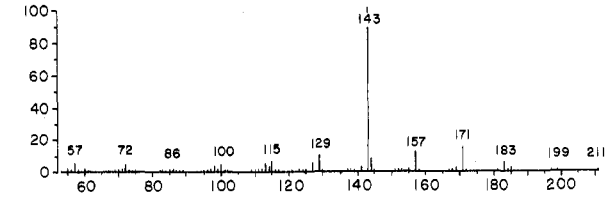


Figure 3. Mass spectrum (CI-methane) of 5-ethyl-5-methylhydantoin (peak 2, Figure 1).

observable for peak 6 in the chromatogram (Figure 1). Although a number of dialkylhydantoins give m/e 114 fragment ions, the occurrence of an intense m/e 86 ion with the 114 ion is unique. Further evidence for the assignment of this peak to 5-methylhydantoin is based on retention index matching and CI mode mass spectrometry described below.

Evidence for the presence of spiropentylhydantoin and spirohexylhydantoin in the condensate water was the observation of peaks in the chromatogram at long retention times (peaks 10 and 11) whose mass spectra matched the standards. In these spectra, ions at m/e 154 and m/e 168 corresponding to the molecular ions of spiropentyl- and spirohexylhydantoin were evident. Fragment ions for the spirohexylhydantoin were found at 139, 125, 113, and 112 as expected (*7*). A fragmentation scheme similar to that written for 1-methylspirohexylhydantoin (*7*) is proposed for spiropentylhydantoin (Figure 2).

Chemical ionization mass spectrometry using methane was successful in confirming the molecular weights of the hydantoins present. The M + 1 ions were especially intense and M + 15, M + 29, M + 41, and M + 43 ions were evident in the hydantoin CI mass spectra (see, for example, the CI spectrum of 5-ethyl-5-methylhydantoin, Figure 3). Peak 3 was identified as 5-isopropyl-5-methylhydantoin on the basis of the M + 1 ion at 157, the M + 29, M + 41, and M + 43 ions present and the retention index, and derivatization data described below. Similarly peaks 4 and 5 were shown to be 5,5-diethylhydantoin and 5-methyl-5-propylhydantoin on the basis of the M + 1 ions at 157 for both peaks. Other con-

Table I. Hydantoins Found in Condensate Water

peak no.	component identified	retention index	molecular ion obsd, EI	ions obsd with CI (CH_4) M + 1	M + 15	M + 29
1	5,5-dimethylhydantoin	142.21	128	129	143	157
2	5-ethyl-5-methylhydantoin	153.54	142	143	157	171
3	5-isopropyl-5-methylhydantoin	158.21		157	171	185
4	5,5-diethylhydantoin	164.08		157	171	185
5	5-methyl-5-propylhydantoin	166.15		157	171	185
6	5-methylhydantoin	170.95	114	115	129	143
7	5-ethyl-5-propylhydantoin	174.69				
8	5-ethylhydantoin	176.56	128			
9	5-butyl-5-methylhydantoin	179.25				
10	spiropentylhydantoin	200	154	155	169	183
11	spirohexylhydantoin	207.57	168	169		

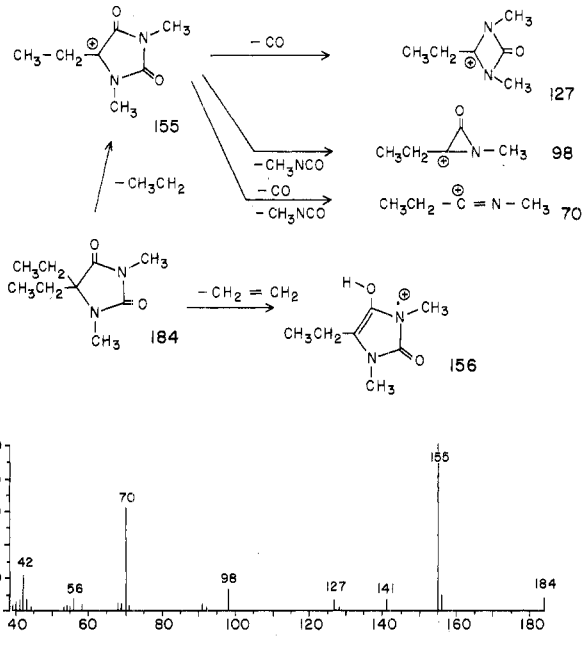
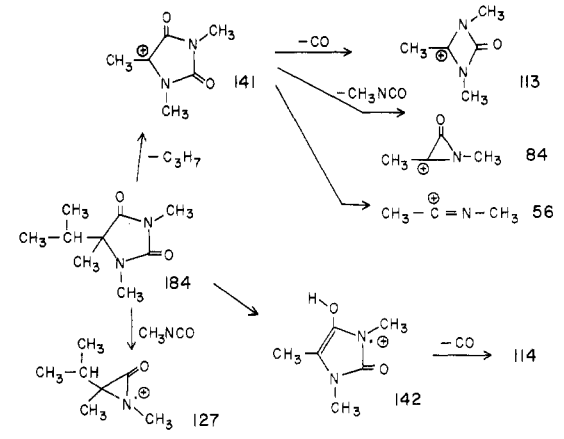


Figure 4. Mass spectrum (EI) and fragmentation scheme for 5,5-diethyl-1,3-dimethylhydantoin.



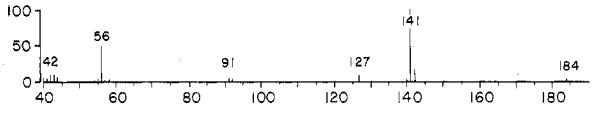


Figure 5. Mass spectrum (EI) and fragmentation scheme for 5-isopropyl-1,3,5-trimethylhydantoin.

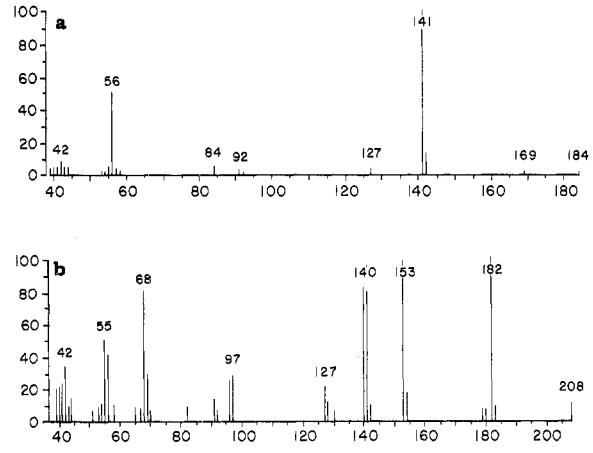


Figure 6. Mass spectrum (EI) of 5-propyl-1,3,5-trimethylhydantoin (a) and 1,3-dimethylspiropentylhydantoin (b).

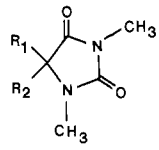
firmations are shown in Table I.

In order to obtain confirmatory molecular ion data for the higher molecular weight dialkylhydantoins, the hydantoins from the condensate water were converted to the 1,3-dimethyl (*N,N'*-dimethyl) derivatives with methyl iodide, and GC/MS data (EI mode) were obtained with a methylphenylsilicone bonded phase (DB5) fused silica capillary column with on-column injection. With this nonpolar column, good peak shapes were obtained for the dimethyl derivatives of the hydantoins although underivatized hydantoins and monomethyl derivatives gave very broad unsymetrical peaks. The order of retention of the 1,3-dimethyl derivatives was different from the underivatized hydantoins; the 5,5-diethyl-1,3-dimethylhydantoin precedes the 5-isopropyl-3,3,5-trimethylhydantoin in eluting from the nonpolar column. The 1,3,5-trimethylhydantoin comes off just after the 1,3,5,5-tetramethylhydantoin and the 1,3-dimethyl-5-ethylhydantoin just after the 5-ethyl-1,3,5-trimethylhydantoin. Molecular ions were observed for the smaller peaks in the chromatogram (Table II). With most components, the most intense ion in the spectrum corresponded to the fragment produced by loss of the larger alkyl substituent at the 5-position.

The base peak for the 5,5-diethyl-1,3-dimethylhydantoin was at *m/e* 155 (Figure 4), consistent with the expected loss of an ethyl radical. Further fragmentation of the 155 ion produced the ions observed at *m/e* 127, 98, and 70 by loss of CO, CH_3NCO, and CO plus CH_3NCO, respectively, in agreement with the fragmentation pattern observed with the 1,3,5,5,-tetramethylhydantoin (*6*). In addition, the McLafferty rearranged ion at 156 was found. A low abundance ion at 128 could have resulted from loss of CO from the 156 ion.

The mass spectrum of the 5-isopropyl-1,3,5-trimethylhydantoin (Figure 5) followed much the same pattern. The base peak (*m/e* 141) resulted from loss of propyl radical. Cleavage of the hydantoin ring of the 141 ion by loss of CH_3NCO and CO plus CH_3NCO gave the ions at *m/e* 84 and

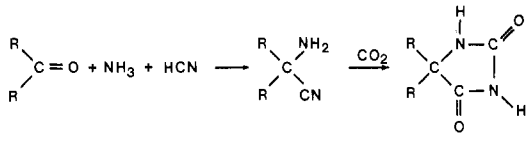
Table II. Ions Observed for 1,3-Dimethylhydantoins[a]



	$R_1 = CH_3$ $R_2 = CH_3$	CH_2CH_3 CH_3	$CH_2CH_2CH_3$ CH_3	$CH(CH_3)_2$ CH_3	CH_2CH_3 CH_2CH_3	CH_3 H	CH_2CH_3 H
M	156	170	184	184	184	142	156
M − 15	141	155	169			127	
M − 29	127	141			155		127
M − R_1 (base)	141	141	141	141	155	127	127
M − R_1 + 1	142	142	142	142	156		128
M − R_2	141	155	169		155	141	155
M − 57	99 w	113 w	127 w	127 w	127		
M − 85	71 w	85 w				57	71
M − 84	70 w	84 w				56	70
M − R_1 − 28	113 w	113 w			127		
M − R_1 − 57	84 w	84 w	84 w	84 w	98	70	70
M − R_1 − 85	56	56	56	56	70	42	42
M − R_1 − 86	55 w	55 w	55 w	55 w	69 w	41	41

[a] Neutral masses lost: 28, CO; 57, NCH_3CO; 85, NCH_3CO + CO; 86, NCH_3CONH_3. w = low intensity.

Scheme I



56. Loss of CH_3NCO from the molecular ion (*m/e* 184) gave the 127 ion. Rearrangement of the molecular ion with loss of propylene gave the ion at 142, as in the case of the 5,5-diethylhydantoin derivative described above.

5-Propyl-1,3,5-trimethylhydantoin showed an ion at *m/e* 169 in the spectrum resulting from loss of methyl and the base peak at 141 from loss of propyl radicals (Figure 6a). Ions were found at 142, 127, 84, and 56 in common with the mass spectrum of the isopropylmethyl derivative. The 1,3-dimethyl derivatives of the 5-methyl- and 5-ethylhydantoins were recognized by their molecular ions at *m/e* 142 and 156 and base peaks at 127 for both.

The mass spectrum of the 1,3-dimethylspiropentylhydantoin (Figure 6b) differs significantly from that of the other dimethyl derivatives in that the molecular ion (*m/e* 182) is intense. Most of the fragment ions observed can be accounted for by a process similar to that shown in Figure 2 for the underivatized spiropentylhydantoin.

A retention index system based on the two reference standards, 1-methylhydantoin and spiropentylhydantoin, was utilized for the identification of hydantoins by retention data comparisons. Spiropentylhydantoin was found in significant amounts in all condensate samples and has a long retention time. 1-Methylhydantoin has not been found in any condensate samples; however, it has been added as an internal standard for the quantitation of the organic species present in the condensate water. These compounds were assigned the reference values 200 and 100, respectively, and the van den Dool and Kratz formula (*8*) was used for the retention index calculation.

Although the two very tiny peaks in the FID chromatogram (peaks 7 and 9) have retention indexes which match those of 5-ethyl-5-propylhydantoin and 5-butyl-5-methylhydantoin, their concentration was too small to be able to get mass spectral data for confirmation of the structures.

The hydantoins are believed to have formed by reaction of ketones and aldehydes with ammonia, carbonate, and cyanide, all of which are present in the condensate water (Scheme I). The temperatures of the spray washer and tar water separator were sufficiently high (>50 °C) for the synthesis of hydantoins via this reaction (*9, 10*). Analysis of water frozen immediately after condensation of gasifier effluent from a side stream showed only trace amounts of hydantoins present.

The same hydantoins were found in samples from various runs of the gasifier using Indian Head coal although the concentrations of hydantoins in the condensate water varied somewhat because of different operating conditions. The concentrations or relative proportions might be expected to change if another coal were used or the operating conditions were changed significantly. Although ketones were not studied specifically, the distribution of major compound classes was shown to vary with the moisture and ash-free carbon content of the coal used in gasification (*11*). The residence times and temperatures of water in the tar–water separator or spray washer may be expected to affect the amount and rate of synthesis of hydantoins from the ketones.

Since hydantoins are well-known as hypnotics (*10*), anticonvulsants (*10*), and suspected carcinogens (*12*), their analysis and removal from the condensate water are important in a commercial gasification plant. This removal cannot be carried out by solvent extraction, cooling tower, or biological treatment; hence research is under way for ascertaining the most effective method for the complete removal of hydantoins from condensate water. A quantitative analytical method involving direct injection of the condensate water samples on a polar capillary column will be published separately.

Registry No. 5,5-Dimethylhydantoin, 77-71-4; 5-ethyl-5-methylhydantoin, 5394-36-5; 5-isopropyl-5-methylhydantoin, 5455-35-6; 5,5-diethylhydantoin, 5455-34-5; 5-methyl-5-propylhydantoin, 5455-36-7; 5-methylhydantoin, 616-03-5; 5-ethyl-5-propylhydantoin, 85320-28-1; 5-ethylhydantoin, 15414-82-1; 5-butyl-5-methylhydantoin, 6326-76-7; spiropentylhydantoin, 699-51-4; spirohexylhydantoin, 702-62-5; water, 7732-18-5.

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Critical Micelle Concentration Determination of Nonionic Detergents with Coomassie Brilliant Blue G-250

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A spectrophotometric assay for critical micelle concentration determination of nonionic detergent solutions is described. The assay utilizes the same dye, Coomassie Brilliant Blue G, and conditions as for the direct protein-dye binding assay.

The use of nonionic detergents has become important to the study of membrane protein structure and function. These detergents differ in their chemical structure and biological interaction with proteins (*1*, *2*). The extent of protein–detergent interaction can often be correlated with the hydrophile–lipophile balance (HLB) (*3*) or critical micelle concentration (CMC) of the detergent (*4*). The HLB for most detergents can be obtained from the literature (*5*), but the CMC usually must be determined empirically (*6*).

CMC determinations are usually made on the basis of a sharp change in the colligative properties, surface tension, solubilization of a hydrophobic dye, e.g., Orange OT, or conductivity of a detergent solution with respect to detergent concentration (*6*). In this paper, we describe an extrapolative assay for CMC determination based on the change in absorption spectrum of Coomassie Brilliant Blue G (CBBG). The CBBG absorption spectrum changes as a function of the HLB of a series of detergents or the micelle concentration of a detergent solution. The assay utilizes the same conditions and reagents established for a widely used method for protein determination (*7*, *8*). This provides a rapid, simple assay for detergent characteristics in solution.

EXPERIMENTAL SECTION

Reagents. Coomassie Brilliant Blue G-250 (CBBG), sodium dodecyl sulfate, and bovine serum albumin (BSA) were obtained from Sigma. The Triton detergents were kindly provided by Jan R. Gelfand, Rohm and Haas, Inc. The other detergents Emulgen 911 and Emulgen 913 (Kao-Atlas) and Lubrol PX and NP 40 (Shell) were obtained from John Chiang, Northeastern Ohio Universities College of Medicine (*9*). All other reagents were standard reagent grade.

Preparation of Solutions. The CBBG reagent was prepared as described by Bradford (*7*) or alternatively, as obtained from Bio-Rad (*8*). Final concentration of CBBG in the reagent was 0.01% (w/v) and in the assay was 40 ppm (w/v). The detergents were made up as 10% (w/w) stock solutions in deionized water.

CMC Assay. The assay procedure is as described by Bradford (*7*) for protein concentration determination. Briefly, varying concentrations of detergent solutions were added in a volume of 0.2 mL to 4.8 mL of the CBBG reagent. Final detergent concentration was based on the total 5 mL volume. The solution was mixed briefly and an absorption spectrum was obtained against a water blank. Alternatively, only absorptions at 470 nm (A_{470}) and 620 nm (A_{620}) were obtained. The A_{470} and A_{620} were plotted independently against detergent concentration. The CMC can readily be determined from this plot.

RESULTS

Coomassie Brilliant Blue G-250 (CBBG) has two major absorption peaks with maxima at 655 nm and 465 nm when prepared as the protein-dye binding assay reagent. Upon binding to protein, a change in the absorption spectrum for CBBG occurs. The peak at 655 nm shifts to 595 nm with a decrease in the peak at 465 nm. Protein determinations can thus be made by measuring the A_{595} of the resulting blue solution. Upon mixing of the dye with organic solvents, such as 2-propanol or chloroform, a single absorption peak with maximum at 615 nm is observed (Figure 1). Low concentrations of Triton X-100, up to 0.008% (approximately 0.13 mM) caused no change in the absorption spectrum of the CBBG reagent. However, increasing the concentration beyond 0.010% (approximately 0.15 mM) caused a concomitant increase in the A_{620} with a decrease in A_{470}. The resultant spectra, at detergent concentrations greater than 0.02%, resemble the spectrum of CBBG in the organic solvent (Figure 2). Similar concentration-dependent changes in CBBG spectra were observed for other nonionic detergents. These results indicate that at high concentrations of nonionic detergent, which correspond to a high detergent micelle concentration with respect to monomer concentration, the dye is completely sequestered within a hydrophobic environment, the micelle interior. This spectral change was not observed for ionic detergents such as sodium dodecyl sulfate or sodium deoxycholate. For these ionic detergents increase in absorption of the CBBG with increasing detergent concentration occurs without a change in the absorption spectrum suggesting that the interaction between CBBG and these detergents differs from that with nonionic detergents.

Correlation with HLB. Since incorporation of CBBG into a hydrophobic environment alters the spectrum of the dye, the relationship between hydrophobicity of a series of Triton X and Triton N detergents, differing in their HLB number (*10*), and the A_{620} and A_{470} of CBBG was investigated. As can be seen in Figure 3, there is an inverse nonlinear relationship between the HLB and the absorption of the detergent–CBBG solution.

Determination of CMC. Since there is a correlation between the HLB of a detergent and its CMC, and for the Triton

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